East African Scholars Journal of Medical Sciences
Abbreviated Key Title: East African Scholars J Med Sci
ISSN 2617-4421 (Print) | ISSN 2617-7188 (Online) |
Published By East African Scholars Publisher, Kenya

OPEN ACCESS

Volume-2 | Issue-12| Dec -2019 | DOI: 10.36349/EASMS.2019.v02i12.008

Original Research Article

The Intrinsic Coagulation Pathway: Early Activation in Paroxysmal Atrial Fibrillation

Mariya Negreva[1*], Krasimira Prodanova[2], Katerina Vitlianova[3] and Christiana Madjova[4]

[1]Associate professor at Department of Cardiology, Medical University of Varna, First clinic of cardiology, University Hospital "St. Marina"-Varna, Varna, Bulgaria
[2]Professor at Faculty of Applied Mathematics and Computer Science, Technical University of Sofia, Sofia, Bulgaria; address: 8 Kl. Ohridski blv., Sofia 1000, Bulgaria
[3]Associate professor at Clinic of Cardiology, SecondCity Hospital of Sofia, Sofia, Bulgaria; address: 120 Hr Botev str., Sofia 1202, Bulgaria
[4]Assistant professor at Department of Conservative dentistry and Oral Pathology, Faculty of Dental Medicine, Medical University-Varna, Varna, Bulgaria; address: 84 Tz Osvoboditel str., Varna, Bulgaria

*Corresponding Author
Mariya Negreva

Abstract: **Introduction** Coagulation factor IX (FIX) is considered a prothrombotic risk factor. Its increased plasma levels and activity are associated with a significantly increased risk of arterial and/or venous thrombosis. The indicator has been poorly studied in atrial fibrillation (AF).**Aim** To examine FIX as indicator of coagulation activity in the early hours (up to 24 hours) of the clinical manifestation of paroxysmal atrial fibrillation (PAF).**Material and methods** 51 non-anticoagulated patients (26 men, 25 women, mean age 59.84±1.60) with PAF and 52 volunteer controls (26 men, 26 women, mean age 59.50±1.46 years) matched in gender, comorbidities, and treatment were selected for the study. FIX plasma coagulation activity was examined using a photometric test.**Results** All patients were hospitalized between the 2nd and 24th hours after the arrhythmia onset (mean 8.14±0.74 hours). PAF group showed higher FIX activity relative to the control group (170.43%±6.62% vs 117.72%±5.95%, $p<0.001$), independent of age and BMI ($p>0.05$), and slightly dependent on PAF duration ($r=0.38$, $p<0.05$). Male gender (186.82%±8.98% vs 154.70%±8.38%, $p<0.05$) and high embolic risk (CHA_2DS_2-VASc score≥2) (156.03%±8.62 vs 184.31%±8.99%, $p<0.05$) were associated with higher FIX activity. **Conclusion**FIX activity is elevated even in the first twenty-four hours of the clinical manifestation of PAF. This gives reason to believe that the intrinsic coagulation cascade pathway is activated, which determines the early expression of PAF as a procoagulant state. At the same time, it is a prerequisite for FIX to become a new target for AF anticoagulant therapy with good antithrombotic efficacy and reduced bleeding risk.

Keywords: atrial fibrillation, coagulation, intrinsic pathway, coagulation factor IX.

INTRODUCTION

The classical coagulation cascade model was proposed in 1964 by Macfarlane and Davie and Ratnoff (Macfarlane RG, 1964; Davie EW *et al*., 1964). Despite its weaknesses and inability to explain major phenomena in the *in vivo* hemostasis, the model was accepted by the clinical community for more than 50 years. It divides the coagulation into an extrinsic pathway (activated by elements external to the blood) and an intrinsic pathway (initiated by blood components), which meet in a final common pathway with factor X (FX) activation. The extrinsic pathway originates from factor VII (FVII), which associated with its co-factor, tissue factor (TF), activates FX. In the intrinsic pathway, FX activation occurs after sequential activation of factor XII (FXII), factor XI (FXI), and factor IX (FIX), initiated when blood contacts surfaces containing a negative electrical charge.

In recent years, the drawbacks of the cascade model have been clearly defined. For example, FXII deficiency significantly prolongs the activated partial thromboplastin time without causing bleeding (Girolami A *et al*., 2004). At the same time, FIX deficiency leads to hemophilia B and the development of serious clinical bleeding (Vine AK, 2009). The classical model could not explain why the extrinsic coagulation pathway does not compensate for the

Quick Response Code

Journal homepage:
http://www.easpublisher.com/easjms/

Article History
Received: 25.11.2019
Accepted: 06.12.2019
Published: 19.12.2019

function of the intrinsic pathway. The question of the close relationship and complementary action of the two coagulation pathways arose. The key role of FIX in the activity of the coagulation cascade as a whole and its participation in the thrombus formation process was outlined.

Clinical and experimental studies have shown a link between increased FIX levels and activity and various thrombotic complications, which supported the idea of the crucial role of the coagulation factor in the thrombus formation process. For example, infusion with purified FIXa in rats caused local and disseminated thrombosis (Gurevich V *et al*., 1979). The blocking of FIXa coagulation activity stopped thrombus formation, including intra-arterial coronary thrombosis in *in vitro* studies (Benedict *et al*., 1991). Clinical studies have shown a direct correlation between the levels of prothrombin fragment 1 + 2 and the thrombin-antithrombin III complex (indicators of the final steps in the coagulation cascade) with FIX levels and activity (Lowe G *et al*., 1997).

Elevated FIX levels have been described as a risk factor for deep vein thrombosis. They are associated with a two- to three-fold increased risk of venous thrombosis, regardless of other prothrombotic risk factors (Lowe G *et al*., 2000). At the same time, they are also a relapse predictor, which gave reason to believe that FIX is directly related to venous thrombosis. Elevated FIX levels were also found in acute coronary syndrome. In some studies, unstable angina and acute myocardial infarction have been associated with high FIX plasma concentrations (Minnema MC *et al*., 2000). A correlation was found between increased FIX activity and stroke and transient ischemic attack rates (Heikal MN *et al*., 2013). In experimental conditions, the prothrombotic effect of tissue factor-bearing particles is directly determined by FIX activity, which has led to the recognition of its role in the process of thrombus formation in malignancies (Tornoem GW *et al*., 2013).

Studies with undoubted results show that FIX is key to thrombus formation. Evidence of this are the results of both *in vitro* experiments and animal models. Increased FIX levels are associated with increased incidence of venous and arterial thrombotic events in clinical practice. This gives reason for FIX to be investigated in other diseases with hemostatic changes, such as atrial fibrillation.

Aim

To examine FIX as an indicator of coagulation activity in the early hours (up to 24 hours) of the clinical manifestation of paroxysmal atrial fibrillation (PAF).

MATERIAL AND METHODS

Study Population

The study was conducted in the Intensive Cardiology Unit of the First Cardiology Clinic at the University Hospital "St. Marina"- Varna for the period 10.2010. - 05.2012г. after approval by the local ethics committee (9/14.10.2010) and in accordance with the Declaration of Helsinki (WMA 2008).

Fifty-one patients (26 men and 25 women) with a mean age of 59.84±1.60 years (31-77 years) were included in the study. They were sequentially selected from a total of 338 patients >18 years of age, hospitalized for PAF with an onset of the episode <24 hours before hospitalization.

A control group of 52 volunteers (59.50±1.46 years; 26 men and 25 women) was formed by screening 169 volunteers without previous anamnestic or electrocardiographic AF data.

The two groups were similar in terms of some indicators that may influence FIX activity in order to equalize their effects on the results in patients and controls. Such indicators were gender, age, BMI, deleterious habits, comorbidities and treatment.

Each participant was enrolled in the study after signing informed consent.

The following inclusion and exclusion selection criteria were used to select the participants.

Inclusion Criteria:

1. Ability to clearly define the onset of arrhythmia, continuing at the time of hospitalization;
2. Lack of exclusion criteria.

Exclusion Criteria:

1. cardiovascular diseases: ischemic heart disease, heart failure, high-grade and / or uncontrolled hypertension, moderate or severe acquired valve defects, cardiomyopathy, implanted device for the treatment of rhythm-conduction disorders, inflammatory heart disease, congenital heart diseases;
2. otherdiseases - kidneyorliverfailure, inflammatory and/or infectious diseases, neoplastic and autoimmune diseases, chronic pulmonary insufficiency, endocrine disorders (except for non-insulin dependent, well-controlled DM type 2); previous thromboembolic incidents, bleeding diathesis, miscarriages (for women);
3. intake of hormone replacement therapy, contraceptives, oral anticoagulants or antiplatelet drugs, pregnancy, systemic intake of analgesics (incl. NSAIDs), obesity with BMI >35;
4. unsuccessful restoration of sinus rhythm with drugs (propafenone) (for the patient group)

Sample Collection and Laboratory Procedures

Blood samples were collected once from each study participant: in patients, immediately after hospitalization; in controls, during outpatient examinations. Blood was collected in coagulation 3.2% sodium citrate (VACUETTE, Greiner Bio-One North America, Inc), centrifuged at 2500g for 15 min, and the resulting plasma was pipetted and stored at -20°C for a maximum of 1 month.

Each indicator was determined twice and the mean was taken into account when calculating the results.

FIX coagulation activity was determined photometrically using a one-stage method based on Activated Partial Thromboplastin Time (aPTT) (Factor IX Deficient Plasma, immunads. Technoclone, Austria).

Statistical Analysis

All analyses were conducted using STATISTICA 13.3.0, StatSoft Inc, and USA.

Continuous variables were expressed as mean ± standard error of the mean (SE) and categorical variables were expressedas percentage of the total group. Normality of distribution was assessed by the Kolmogorov-Smirnoff test. Two-tailed Student's t-test for independent samples was used to compare quantitative variables. Fisher's exact or Pearson's chi-square tests were used to compare categorical variables and occurrence frequency. Values $p<0.05$ were adopted for statistically significant.

Linear regression analysis was used to analyze the strength of the association between the dependent variable FIX coagulation activity and the continuous explanatory variables age, BMI and PAF duration (time spent in AF until hospitalization).

Associations between FIX activity in PAF patients and the categorical independent variables sex and CHA_2DS_2–VASc score were determined by an analysis of variance (ANOVA). Variables showing a level of association $p<0.05$ were considered prognostic.

RESULTS

The patient and control groups did not differ significantly in age, gender, comorbidities and treatment, deleterous habits, and BMI ($p>0.05$) (Table 1). Transthoracic echocardiography indicators also did not show significant differences ($p> 0.05$) (Table 2).

PAF-episode onset analysis showed that patients were hospitalized between the 2nd and the 24th hour of the onset of arrhythmia, most often by the 5th hour. The average duration of episodes until hospitalization was 8.14±0.76 hours.

Table 1. Clinical characteristics of the participants

	Patients with PAF	Control group	P values
Number of participants	51	52	p=0.89
Mean age (years)	59.84±1.60	59.50±1.46	p=0.87
Men/Women	26/25	26/26	p=1/p=0.93
Accompanying diseases			
Hypertension	37 (72.54%)	34 (65.38%)	p=0.44
Diabetes mellitus type 2	3 (5.88%)	2 (3.84%)	p=0.62
Dyslipidemia	4 (7.84%)	3 (5.77%)	p=0.69
Medicaments for Hypertension and Dyslipidemia			
Beta blockers	19 (37.25%)	17 (32.69%)	p=0.62
ACE inhibitors	15 (29.41%)	14 (26.92%)	p=0.78
Sartans	11 (21.57%)	9 (17.31%)	p=0.58
Statins	4 (7.84%)	3 (5.77%)	p=0.69
Deleterious habits			
Smoking*	8(15.69%)	7(13.46%)	p=0.75
Alcohol intake**	7(13.72%)	6(11.53%)	p=0.74
BMI (kg/m^2)	23.85±0.46	24.95±0.45	p=0.09
CHA_2DS_2–VASc score			
Number of patients with score < 2	25	No score	
Number of patients with score ≥ 2	26		

**no more than half a pack of cigarettes weekly.*
***no more than two drinks weekly.*

Table 2. Echocardiographic parameters of the participants

	Patients with PAF	Control group	P values
Echocardiographic indicators			
LVEDD (mm)	52.57±0.58	52.29±0.57	p=0.73
LVESD (mm)	34.43±0.56	34.73±0.48	p=0.69
EF (%)	62.98±0.70	61.54±0.58	p=0.12
IVS (mm)	10.37±0.23	9.92±0.26	p=0.20
PW (mm)	10.24±0.21	9.73±0.28	p=0.16
LA volume (ml/m²)	22.81±0.45	23.82±0.48	p=0.13
RVEDD (mm)	30.54±1.58	29.17±1.52	p=0.18

Plasma Activity of FIX

Significantly higher FIX activity was observed in PAF patients compared to controls in sinus rhythm (170.43%±6.62% vs 117.72%±5.95%, p<0.001; Fig 1).

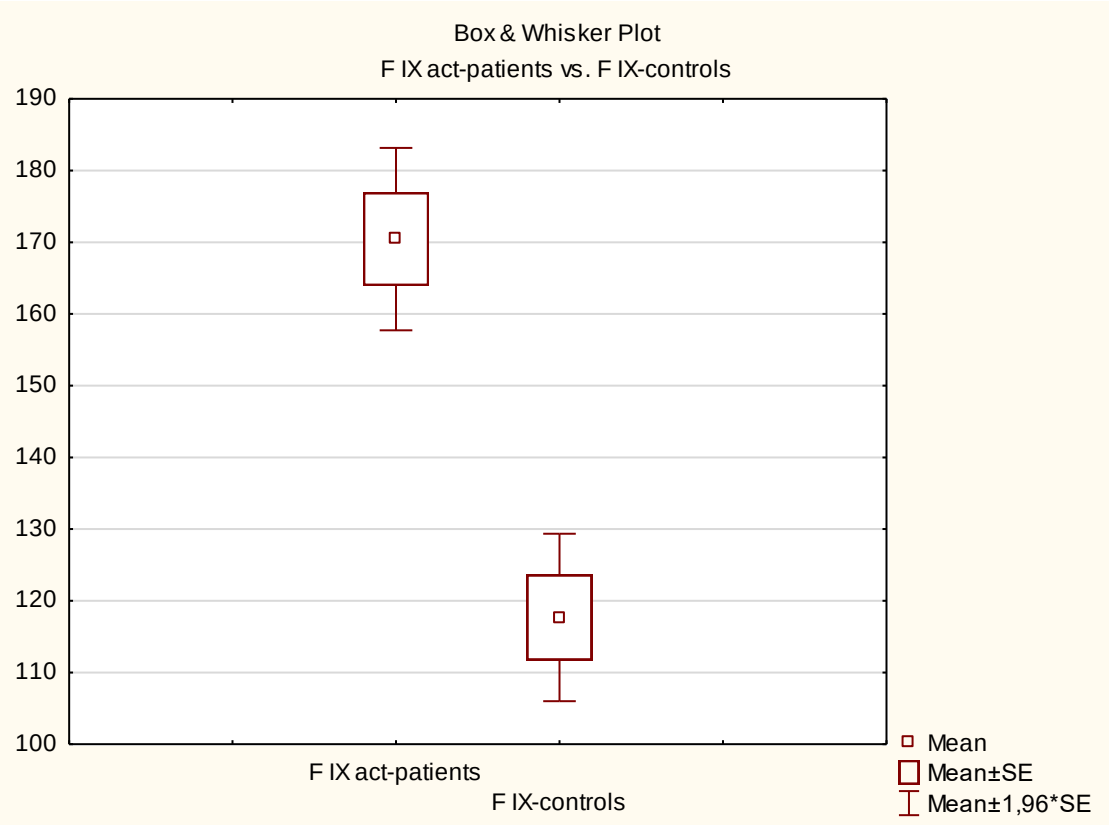

Figure 1. FIX coagulation activity in patients with PAF and controls in sinus rhythm (FIX act – factor IX activity).

Linear regression analysis showed that age and BMI were not predictive variables for coagulation activity of FIX (r=0.07, p>0.05; r=0.11, p>0.05, respectively) There was a statistically significant but weak correlation between duration of PAF episode (time spent in AF until hospitalization) and FIX activity (r = 0.38, p<0.05; Fig. 2). ANOVA analysis showed a significant difference in FIX activity between men and women in PAF (186.82%±8.98% vs 154.70%±8.38%, p<0.05), as well as between patients with low risk (CHA_2DS_2 -VASc score<2) and high risk (CHA_2DS_2 -VASc score≥2) (156.03%±8.62 vs 184.31%±8.99%, p<0.05).

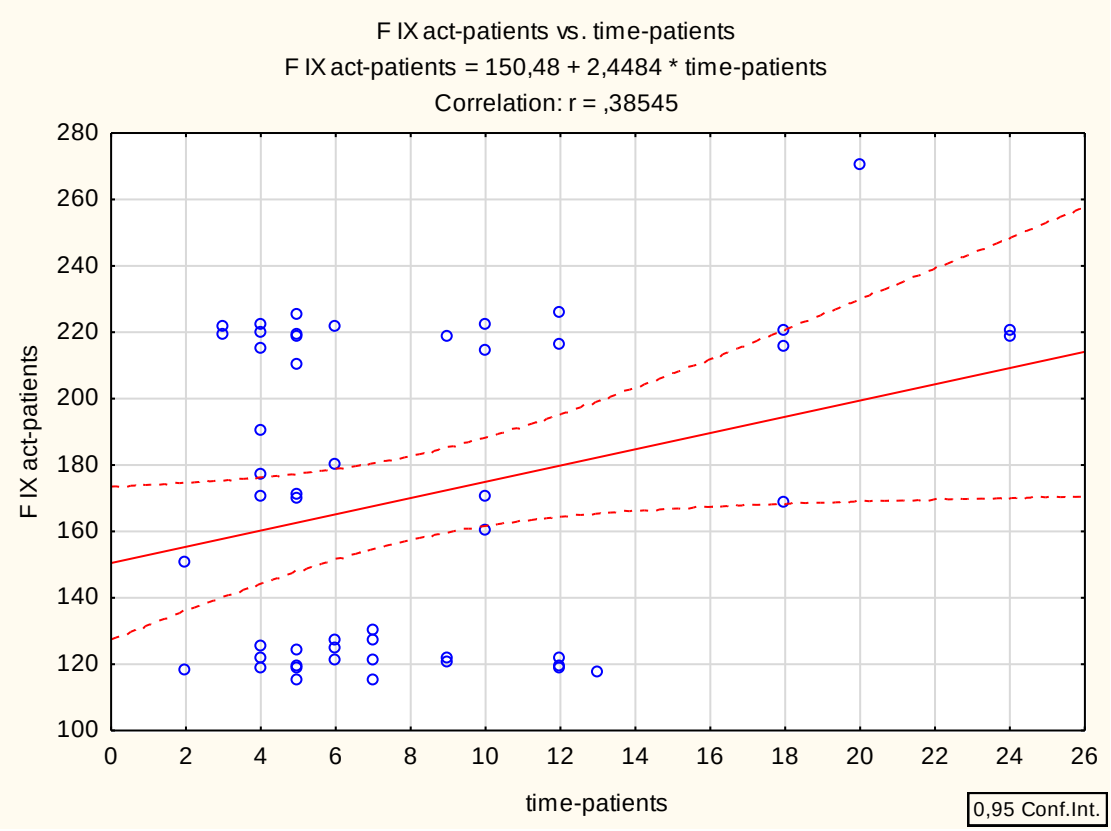

Fig. 2. Correlation between FIX activity and PAF duration (time-patients – time patients spent in PAF)

DISCUSSION

FIX is a key coagulation factor. According to classical theory, it is activated by FXIa upon initiation of the coagulation cascade via the intrinsic coagulation pathway (Macfarane RG, 1964; Davie EW *et al*., 1964). In this sense, classical theory relates the activity of coagulation factors to the presence of artificial surfaces in plasma, leading to conformational changes and FXII activation. Modern hemostasis concepts define FIX as a major molecular coagulation cascade, directly responsible for the propagation stage of thrombus formation in any procoagulant stimulation, regardless of its nature and origin (Wheeler AP *et al*., 2016; Mackman N, 2009). According to the current cell-based coagulation model, the potent TF/FVIIa protease complex formed upon activation of FVII by TF, activates not only FX but also turns non-active FIX into active (Smith SA, 2009). Within the tenase complex linked to FVIIIa on the platelet surface, it is responsible for the coagulation-critical formation of FXa, which generates sufficient thrombin to participate in the thrombus-forming and clot-propagation phase. Thus, the initiation phase of the coagulation process is related to the activity of the extrinsic pathway, and retention and propagation – to the intrinsic pathway.

FIXa is a molecule which presents the close relationship between the two pathways of coagulation cascade activation. Defined as an intrinsic pathway factor, it simultaneously successfully realizes the TF/FVIIa procoagulant effect, enhancing FX activation almost 50 times more effectively than direct activation by the FVIIa/TF complex (Lawson JH *et al*., 1991).

The importance of FIX, outlined by the new concept of hemostasis, is of interest to AF patients. Increased activity of the indicator was found in the permanent forms of the disease against the background of anticoagulant therapy, which led the authors to suggest the molecule as a possible marker of thromboembolic risk in AF (Kusak P *et al*., 2016). Elevated FIXa: antithrombin III complex levels have been measured in PAF patients outside the arrhythmic episode during sinus rhythm (Hobbelt A *et al*., 2016).

The results of our study show a significantly increased FIX activity in the early hours (up to 24 hours) of the clinical manifestation of PAF ($p < 0.001$; Fig. 1). The clearly defined role of FIX in the coagulation process according to modern concepts of hemostasis, gives us reason to interpret our results as a sign that the initiation of the coagulation cascade is indisputably present in the studied PAF population. High FIX activity is a prerequisite for generating a significant amount of thrombin, responsible for fibrin formation and completing the coagulation process with clot formation. PAF manifestation is associated with high FIX activity, which predetermines the possibility of thrombus formation even in its early clinical presentation. This prothrombotic condition is independent of age and BMI ($p<0.05$), shows slight correlation with PAF duration ($r=0.38$, $p<0.05$) and

depends on sex and CHA_2DS_2 – VASc score ($p<0.05$). Male gender is associated with higher FIX activity. Higher embolic risk (calculated by CHA_2DS_2 – VASc score), as expected, corresponds to higher values of FIX coagulation activity.

The obtained results have important clinical implications. Not only do they show the intimate mechanisms of impaired hemostasis, but they suggest new therapeutic options. Direct oral anticoagulants, used to prevent thromboembolic events in AF patients, act by blocking the final common pathway of the coagulation cascade (Ghanima W *et al*., 2013). Target of dabigatran etexilate thrombin, rivaroxabane and apixaban block the activated FX. Their benefits and their good anticoagulant efficacy are inevitably associated with risk of bleeding. According to some epidemiological data, the incidence of significant and insignificant clinical bleeding reaches up to 15% in the general population of AF patients (Granger CB *et al*., 2011; Patel MR *et al*., 2011).

This is a prerequisite for finding new molecules with lower bleeding potential. Reduction of 99-50% of FIX levels (observed in patients with mild and moderate hemophilia B and disease carriers) has been found to be associated with reduced thrombotic risk at an absolutely acceptable risk of bleeding (Darby SC *et al*., 2007). Severe and spontaneous bleeding episodes were observed in patients with residual activity <1%, and its increase to 1-6% of normal activity leading to their disappearance (Nathwani AC *et al*., 2014). FIXa is one of the coagulation cascade proteases via which the two pathways complement their procoagulant effect. Unlike FXa, it is relatively stable and has the ability to switch to activated platelets, which are directly related to the amplification and propagation phases of the coagulation process. Its key procoagulant activity, in addition to an acceptable bleeding profile, translates our results into opportunities for new approaches in anticoagulant treatment. The established significant increase in FIX activity was the reason for FIX to become a new therapeutic target for the prevention of thromboembolic events in AF. We expect the moderate anti-FIXa inhibition to lead to a significant reduction in thrombotic events and a low risk of bleeding.

CONCLUSION

FIX activity is elevated even in the first twenty-four hours of the clinical manifestation of PAF. This gives reason to believe that the intrinsic coagulation cascade pathway is activated, which determines the early expression of PAF as a procoagulant state. At the same time, it is a prerequisite for FIXa to become a new target for AF anticoagulant therapy with good antithrombotic efficacy and reduced risk of bleeding.

Acknowledgements

The study was not supported by any agency. The authors have no conflicts of interest to declare.

The second author Krasimira Prodanova is totally supported by project DN12/11/20.dec.2017 of the Ministry of Education and Science of Bulgaria for statistical software STATISTICA 13.3.0, StatSoft Inc, USA.

REFERENCES

1. Macfarlane, R. G. (1964). An enzyme cascade in the blood clotting mechanism and its function as a biochemical amplifier. *Nature*, *202*(4931), 498-499.
2. Davie, E. W., & Ratnoff, O. D. (1964). Waterfall sequence for intrinsic blood clotting. *Science*, *145*(3638), 1310-1312.
3. Girolami, A., Zocca, N., Girolami, B., Lombardi, A. M., & Fabris, F. (2004). Pregnancies and oral contraceptive therapy in severe (homozygons) FXII deficiency: a study in 12 patients and review of the literature. *Journal of thrombosis and thrombolysis*, *18*(3), 209-212.
4. Vine, A. K. (2009). Recent advances in haemostasis and thrombosis. *Retina*, *29*(1), 1-7.
5. Gurewich, V., Nunn, T., & Lipinski, B. (1979). Activation of intrinsic or extrinsic blood coagulation in experimental venous thrombosis and disseminated intravascular coagulation: pathogenetic differences. *Thrombosis research*, *14*(6), 931-940.
6. Benedict, C. R., Ryan, J., Wolitzky, B., Ramos, R., Gerlach, M., Tijburg, P., & Stern, D. (1991). Active site-blocked factor IXa prevents intravascular thrombus formation in the coronary vasculature without inhibiting extravascular coagulation in a canine thrombosis model. *The Journal of clinical investigation*, *88*(5), 1760-1765.
7. Lowe, G. D. O., Rumley, A., Woodward, M., Morrison, C. E., Philippou, H., Lane, D. A., & Tunstall-Pedoe, H. (1997). Epidemiology of coagulation factors, inhibitors and activation markers: The Third Glasgow MONICA Survey I. Illustrative reference ranges by age, sex and hormone use. *British journal of haematology*, *97*(4), 775-784.
8. Lowe, G., Woodward, M., Vessey, M., Rumley, A., Gough, P., & Daly, E. (2000). Thrombotic variables and risk of idiopathic venous thromboembolism in women aged 45-64 years. *Thrombosis and haemostasis*, *83*(04), 530-535.
9. Minnema, M. C., Peters, R. J. G., De Winter, R., Lubbers, Y. P. T., Barzegar, S., Bauer, K. A., ... & Cate, H. T. (2000). Activation of clotting factors XI and IX in patients with acute myocardial infarction. *Arteriosclerosis, thrombosis, and vascular biology*, *20*(11), 2489-2493.

10. Heikal, N. M., Murphy, K. K., Crist, R. A., Wilson, A. R., Rodgers, G. M., & Smock, K. J. (2013). Elevated factor IX activity is associated with an increased odds ratio for both arterial and venous thrombotic events. *American journal of clinical pathology*, *140*(5), 680-685.
11. Tormoen, G. W., Khader, A., Gruber, A., & McCarty, O. J. (2013). Physiological levels of blood coagulation factors IX and X control coagulation kinetics in an in vitro model of circulating tissue factor. *Physical biology*, *10*(3), 036003.
12. World Medical Association. (2001). Ethical principles for medical research involving human subjects. *European journal of emergency medicine: official journal of the European Society for Emergency Medicine*, *8*(3), 221.
13. Wheeler, A. P., & Gailani, D. (2016). The intrinsic pathway of coagulation as a target for antithrombotic therapy. *Hematology/Oncology Clinics*, *30*(5), 1099-1114.
14. Mackman, N. (2009). The role of tissue factor and factor VIIa in hemostasis. *Anesthesia and analgesia*, *108*(5), 1447.
15. Smith, S. A. (2009). The cell-based model of coagulation. *Journal of veterinary emergency and critical care*, *19*(1), 3-10.
16. Lawson, J. H., & Mann, K. G. (1991). Cooperative activation of human factor IX by the human extrinsic pathway of blood coagulation. *Journal of Biological Chemistry*, *266*(17), 11317-11327.
17. Kusak, P., Czarnecka, D., Gissel, M., Plens, K., Butenas, S., & Undas, A. (2016). Activated factor IX, factor XI and tissue factor identify patients with permanent atrial fibrillation treated with warfarin who are at risk of ischemic stroke. *Archives of medical science: AMS*, *12*(5), 1000.
18. Hobbelt, A. H., Rienstra, M., Crijns, H. J., De With, R. R., Dudink, E. A., Ten Cate, H., ... & Van Gelder, I. C. (2016). Hypercoagulability in Anticoagulation-Naive Young Paroxysmal Atrial Fibrillation Patients. *Circulation*, *134*(suppl_1), A18438-A18438.
19. Ghanima, W., Atar, D., & Sandset, P.M. (2013). New oral anticoagulants- a review. *Tidsskr Nor Laegeforen*, 133(18),1940-5.
20. Granger, C. B., Alexander, J. H., McMurray, J. J., Lopes, R. D., Hylek, E. M., Hanna, M., ... & Bahit, M. C. (2011). Apixaban versus warfarin in patients with atrial fibrillation. *New England Journal of Medicine*, *365*(11), 981-992.
21. Patel, M. R., Mahaffey, K. W., Garg, J., Pan, G., Singer, D. E., Hacke, W., ... & Becker, R. C. (2011). Rivaroxaban versus warfarin in nonvalvular atrial fibrillation. *New England Journal of Medicine*, *365*(10), 883-891.
22. Darby, S. C., Kan, S. W., Spooner, R. J., Giangrande, P. L., Hill, F. G., Hay, C. R., ... & Williams, M. (2007). Mortality rates, life expectancy, and causes of death in people with hemophilia A or B in the United Kingdom who were not infected with HIV. *Blood*, *110*(3), 815-825.
23. Nathwani, A. C., Reiss, U. M., Tuddenham, E. G., Rosales, C., Chowdary, P., McIntosh, J., ... & Riddell, A. (2014). Long-term safety and efficacy of factor IX gene therapy in hemophilia B. *New England Journal of Medicine*, *371*(21), 1994-2004.